Chapter 12
Protein Synthesis: Translation of the Genetic Message

SUMMARY

Section 12.1

- Protein translation involves 3 types of RNA and many protein factors.
- Amino acids are activated via enzymes called aminoacyl-tRNA synthetases.
- The formation of a protein takes place in four steps called activation, initiation, elongation, and termination.

Section 12.2

- The genetic code is based on a series of three bases coding for an amino acid.
- The code is nearly universal in all organisms from viruses through humans. The code has no punctuation, meaning the mRNA is read three bases at a time with no spaces in between. The code is non-overlapping as well, meaning that each base is part of only one codon.
- The genetic code was determined by a variety of techniques, such as using synthetic mRNA with known sequences to see what proteins would be translated from them.
- While there are 64 combinations of three bases leading to 64 codons, there are fewer types of tRNA anticodons. This means that standard Watson-Crick base pairing must be broken on occasion. The wobble model of codon-anticodon base pairing shows that some bases at the 5' end of the anticodon of the tRNA can base pair with multiple bases on the codon.
- While there may be many codons for a particular amino acid, not all potential codons are represented equally.

Section 12.3

- Before amino acids can be incorporated into a peptide, they must be activated.
- Amino acids are activated through a reaction with ATP yielding an amino acid bonded to AMP. The AMP-bound amino acid is then reacted with a tRNA to yield an amino acyl tRNA.
- The enzymes responsible for activation are called aminoacyl-tRNA synthetases.

Section 12.4

- The unique and elegant structure of the ribosome allows the binding of aminoacyl-tRNA molecules and mRNA. The ribosome catalyzes the nucleophilic attack of one amino acid upon the next, allowing for protein synthesis
- Protein synthesis begins at an AUG codon on the mRNA. The ribosome and mRNA forms an initiation complex that includes the two main ribosomal subunits, the mRNA, GTP, and three initiation factors, IF1, IF2, and IF3.
- The ribosome locates the correct AUG to start translation by binding to a consensus sequence called the Shine-Dalgarno sequence.
- The first aminoacyl-tRNA bound to the ribosome carries N-formyl-methionine, and it is initially bound to the P-site of the ribosome.

- In chain elongation, the second amino acyl-tRNA binds to the A-site. This amino acid's α-amino group performs a nucleophilic attack on the carbonyl group of the N-formyl-methionine in the peptidyl transfer reaction. In a translocation step, the ribosome then moves one codon leaving a dipeptidyl-tRNA in the A-site and moving the uncharged tRNA to the exit site. The process continues with a new aminoacyl-tRNA entering the P-site. The uncharged tRNA is then ejected from the E-site.
- When the ribosome encounters a stop codon, the chain is terminated in a process requiring GTP and three protein release factors.
- The ribosome is actually a ribozyme. There are no amino acids at the active site where the peptidyl transferase reaction occurs. Specific bases on the rRNA are believed to catalyze the reaction.

Section 12.5

- Eukaryotic translation involves many more protein factors than the corresponding translation in prokaryotes
- Both the 5'-Cap and the 3' poly-A tail are involved in orienting the ribosome close to the correct AUG used as the start codon. There is no Shine-Dalgarno sequence in eukaryotic mRNA.
- Once bound, the ribosome moves down the mRNA scanning for the correct AUG until it finds one that is in the correct context, which is identified by a small mRNA sequence around the AUG called a Kozak sequence.
- Eukaryotic chain elongation is similar to the prokaryotic counterpart. With chain termination, there is only one release factor that binds to all three stop codons.
- It has recently been found that there is some coupled transcription and translation in the nucleus of eukaryotic cells.
- It has also been recently found that in certain circumstances found in the immune system, AUG is not the start codon, rather CUG, leaving leucine as the N-terminal amino acid.

Section 12.6

- Proteins are usually modified after their initial translation.
- Protein modification includes removal of the N-formyl-methionine from prokaryotic proteins, cleavage of specific amino acids, and addition of signal sequences.
- Non-protein components can be added to some proteins, such as the heme group added to hemoglobin.
- Proteins must also be folded properly into their correct form. Protein molecules called chaperones help many proteins fold into their correct form.
- It was recently found that in some cases, the ribosome itself acts as its own chaperone and that proteins translated apart from ribosomes fold differently than proteins translated with ribosomes.

Section 12.7

- Proteins are degraded in subcellular organelles, such as lysosomes, or in macromolecular structures called proteasomes.

- Many proteins are targeted for destruction by being bound to a protein called ubiquitin.
- The nature of the amino acid sequence at the N-terminus is often very important to control of the timing of destruction of a protein.

LECTURE NOTES

As with the material of the previous two chapters, most students will have been introduced to translation in earlier courses, particularly in beginning biology courses, but they are unlikely to have gone into any of the molecular details. since all three processes (replication, transcription, and translation) involve the production of a linear polymer using a template, it is useful to point out similarities and differences among the three. In this manner each topic reinforces the other. Post-translational modification and degradation of proteins are more likely to be new to students, but not necessarily difficult. This chapter will easily require one lecture, and possibly two, dependent upon the level of detail presented.

LECTURE OUTLINE

I. Overview of translation
    A. Amino acid activation
    B. Chain initiation, elongation, and termination
II. The genetic code
    A. Triplet codons
        1. Nonoverlapping
        2. Commaless
        3. Degenerate
        4. Universality
    B. Codon-anticodon pairing and wobble
III. Amino acid activation
IV. Translation in prokaryotes
    A. Ribosomal architecture
        1. Two subunits
        2. Binding sites for two tRNAs
    B. Chain initiation
        1. N-terminus —> C-terminus
        2. 5' —> 3' reading of both DNA & mRNA
        3. N-formylmethionine as first amino acid & AUG start codon
        4. Assembly of initiation complex
        5. Importance of Shine-Dalgarno sequence
    C. Chain elongation
        1. P, A, and E sites
        2. Function of elongation factors
        3. Formation of peptide bond
        4. Translocation
    D. Chain termination
        1. Stop codons
        2. Release factors

3. Disassembly of ribosomal complex

E. Ribosome as a ribozyme
F. Polysomes – coupling of transcription & translation

V. Translation in eukaryotes
A. Chain initiation
1. Eukaryotic initiation factors
2. Importance of 5' cap
3. Ribosomal scanning for start codon – Kozak sequence
4. Assembly of initiation complex
B. Chain elongation
1. Eukaryotic elongation factors
2. Toxins that inhibit prokaryotic, but not eukaryotic, translation
C. Chain termination
1. Single release factor
2. Supressor tRNA
D. Selenocysteine
E. Coupled transcription & translation in eukaryotes?

VI. Posttranslational modification of proteins
A. Removal of N-formylmethionine
B. Leader sequences
C. Additions of cofactors
D. Covalent modification of residues
E. Additions of other groups – carbohydrates, lipids
F. Protein folding

VII. Protein Degradation
A. Proteosomes
B. Ubiquitin

ANSWERS TO PROBLEMS

12.1 Translating the Genetic Message

1. See Figure 12.1.

12.2 The Genetic Code

2. A code in which two bases code for a single amino acid allows for only 16 (4 × 4) possible codons, which is not adequate to code for 20 amino acids.
3. A degenerate code is one in which more than one triplet can specify a given amino acid.
4. In the binding assay technique, various tRNA molecules, one of which is radioactively labeled with ^{14}C, are mixed with ribosomes and synthetic trinucleotides bound to a filter. If the radioactive label is detected on the filter, then it is known that the particular tRNA bound to that triplet. The binding experiments can be repeated until all the triplets are assigned.
5. The wobble base can be uracil, guanine, or hypoxanthine.
6. The codons UAA, UAG, and UGA are the stop signals. These codons are not recognized by any tRNAs, but they are recognized by proteins called release

factors. A release factor not only blocks the binding of a new aminoacyl-tRNA but also affects the activity of the peptidyl transferase, so that the bond between the carboxyl end of the peptide and the tRNA is hydrolyzed.

7. Note that the sequence in the codon of mRNA is reversed because mRNA synthesis is antiparallel.

(a) Position 1 has an intermediate effect. For purine changes, a different amino acid results in all cases. The changes tend to be conservative, with only four of the 16 possible changes leading to hydrophobic-hydrophilic differences. For our purposes, glycine is considered neither hydrophobic nor hydrophilic. The resulting protein would have a better chance of functioning than in a second-base change, but a lesser probability than in a third-base change.

(b) Position 2 is the most informational: a different amino acid results from any change. In this case, however, the chances are high (75%) that the mutation would be a conservative one, with one hydrophobic amino acid replacing another one, so the protein would still have a good chance of functioning. A change involving serine or threonine (25% chance) would alter the polarity but would not introduce a charge on the side chain; the protein might still function.

(c) There is a high probability of a change in the type of amino acid, including differences in charge; the probability of the resulting protein having proper function is considerably lower.

(d) Position 3 is the least informational. There is a high probability of getting the same amino acid. The protein thus has a very good chance of functioning.

8. The concept of wobble specifies that the first two bases of a codon remain the same, while there is room for variation in the third base. This is precisely what is observed experimentally.

9. Hypoxanthine is the most versatile of the wobble bases; it can base pair with adenine, cytosine, or uracil.

10. It is quite reasonable. When codons for a given amino acid have one or two nucleotides in common, a mutation is less likely to give rise to a nonfunctional protein. The survival value of such a feature guarantees its selection in evolution.

11. An ambiguous code would allow for variation in the amino acid sequence of proteins. Consequently, there would be variation in function, including a number of nonfunctional proteins.

12. Variations in the genetic code in mitochondria support the idea of their existence as free-living bacteria early in evolutionary history.

13. A virus that is too lethal will kill its host before having time to be spread to other hosts. With modern science, such a virus would be isolated and destroyed quickly. By being less lethal, a virus can pass from host to host and make it difficult to isolate.

14. The mRNA when translated yields 191 amino acids from the normal reading frame of mRNA. However, then the ribosome encounters a CGU codon, which is a rare codon for arginine (R). Due to the relative lack of tRNA-Arg that matches the rare codon, the ribosome is stalled on the mRNA. This gives the ribosome time to reset to a new frame, called the +1 frame, where it continues translating, yielding the next 61 amino acids that are different compared to standard PA since they are read out of frame.

12.3 Amino Acid Activation

15. The hydrolysis of ATP to AMP and PP_i provides the energy to drive the activation step.
16. Proofreading in amino acid activation takes place in two stages. The first requires a hydrolytic site on the aminoacyl-tRNA synthetase; incorrect amino acids that have become esterified to the tRNA are removed here. The second stage of proofreading requires the recognition site on the aminoacyl-tRNA synthetase for the tRNA itself. The incorrect tRNA does not bind tightly to the enzyme.
17. The following factors ensure fidelity in protein synthesis. Aminoacyl-tRNA formation includes a high degree of enzyme specificity to connect the right amino acid to the right tRNA, proofreading in the formation of some aminoacyl-adenylates, and energy "overkill." Other factors include proper hydrogen bonding of mRNA to the ribosome and between codon and anticodon. (The latter is a relatively slow association, allowing time for mismatches to dissociate before the peptide bond is formed.) The fidelity of protein synthesis is low compared with DNA synthesis, which has proofreading procedures in addition to energy overkill and proper base pairing. The fidelity of protein synthesis is relatively high compared with RNA synthesis, which has only energy overkill and proper base pairing.
18. A separate synthetase exists for each amino acid, and this synthetase functions for all of the different tRNA molecules for that amino acid.
19. The linkage of amino acids to tRNA is as an aminoacyl ester.
20. Proofreading at the activation step allows for selection of both the amino acid and the tRNA. If proofreading took place at the level of codon–anticodon recognition, there would not be a mechanism to ensure that the correct amino acid has been esterified to the tRNA.
21. The overall process of amino acid activation is energetically favored because of the energy contributed by the hydrolysis of two phosphate bonds. Without that input of energy, it would not be favorable.

12.4 Prokaryotic Translation

22. Peptidyl transferase catalyzes the formation of a new peptide bond in protein synthesis. The elongation factors, EF-Tu and EF-Ts, are required for binding of aminoacyl tRNA to the A site. The third elongation factor, EF-G, is needed for the translocation step in which the mRNA moves with respect to the ribosome, exposing the codon for the next amino acid. EF-P is thought to help catalyze the formation of the first peptide bond.
23. The initiation complex in *E. coli* requires mRNA, the 30S ribosomal subunit, fmet-$tRNA^{fmet}$, GTP, and three protein-initiation factors, called IF-1, IF-2, and IF-3. The IF-3 protein is needed for the binding of mRNA to the ribosomal subunit. The other two protein factors are required for the binding of fmet-$tRNA^{fmet}$ to the mRNA-30S complex.
24. Attachment of the 50S ribosomal subunit to the 30S subunit in the initiation complex is needed for protein synthesis to proceed to the elongation phase.

25. The A site and the P site on the ribosome are both binding sites for charged tRNAs taking part in protein synthesis. The P (peptidyl) site binds a tRNA to which the growing polypeptide chain is bonded. The A (aminoacyl) site binds to an aminoacyl tRNA. The amino acid moiety is the next one added to the nascent protein. The E (exit) site binds the uncharged tRNA until it is released from the ribosome.
26. Puromycin terminates the growing polypeptide chain by forming a peptide bond with its C-terminus, which prevents the formation of new peptide bonds (see Figure 12.14).
27. The stop codons bind to release factors, proteins that block binding of aminoacyl tRNAs to the ribosome, and to release the newly formed protein.
28. In the course of protein synthesis, mRNA binds to the smaller ribosomal subunit.
29. The Shine–Dalgarno sequence is a purine-rich leader segment of prokaryotic mRNA. It binds to a pyrimidine-rich sequence on the 16S rRNA part of the 30S ribosomal subunit and aligns it for proper translation, beginning with the AUG start codon.
30. Your friend is mistaken. The hydrogen-bonded regions contribute to the overall shape of the tRNA. Hydrogen-bonded regions are also important in the recognition of tRNAs by aminoacyl-tRNA synthetases.
31. Methionine bound to $tRNA^{fmet}$ can be formylated, but methionine bonded to $tRNA^{met}$ cannot be.
32. Different tRNAs and different factors are involved. Initiation requires IF-2, which recognizes fmet-$tRNA^{fmet}$ but not met-$tRNA^{fmet}$. Conversely, in elongation, EF-Tu recognizes $metRNA^{met}$ but not fmet-$tRNA^{fmet}$.
33. The methionine anticodon (UAC) on the tRNA base pairs with the methionine codon AUG in the mRNA sequence that signals the start of protein synthesis.
34. The fidelity of protein synthesis is assured twice during protein synthesis—the first time during activation of the amino acids and the second time during the matching of the codon to the anticodon on the mRNA.
35.

(a) Activation cycles needed for a protein with 150 AA: 150.
(b) Initiation cycles needed for a protein with 150 AA: 1.
(c) Elongation cycles needed for a protein with 150 AA: 149.
(d) Termination cycles needed for a protein with 150 AA: 1.

36. Four high-energy phosphate bonds per amino acid: two in aminoacyl-tRNA formation, one in elongation with EF-Tu, and one in translocation from the A to the P site, involving EF-G. Forming a peptide bond requires about 5 kcal/mol. This is an expenditure of about 30 kcal/mol peptide bonds. This is the price of low entropy and high fidelity.
37. Not very precisely. Ignoring any editing or proofreading costs, a maximum value can be calculated in terms of high-energy phosphate bonds. We will designate each phosphate bond as ~P. Four are needed per amino acid, and two are needed per ribonucleotide or deoxyribonucleotide. Therefore, four ~P per amino acid × six ~P per codon × six ~P per DNA triplet = 144 ~P per amino acid (approximately 1050 kcal per mole of amino acid). However, the actual value would be much less because of several factors. A single mRNA molecule can be

involved in the synthesis of several to many protein molecules before it is degraded. One gene can be involved in the synthesis of many mRNA molecules, with replication taking place only once per cell generation. In addition, rRNA and tRNA are relatively long-lived and available for repeated protein syntheses.

38. The fact that peptidyl transferase is one of the most conserved sequences in all of biology may indicate that it evolved very early in evolution and that it is so critical for all living organisms that it cannot be modified.
39. The less highly purified ribosome preparations contained polysomes, which are more active in protein synthesis than single ribosomes.
40. At first, peptide-bond formation was catalyzed by RNA. In time, as protein catalysts developed and became more efficient, proteins became an integral part of the ribosome.
41. Electron microscopy can give information about ribosomal structure and function, but X-ray crystallography has given far more detailed information.
42. Because the tRNAs are bound in proximity to each other on the ribosome, the growing polypeptide chain and the amino acid to be added are also close to each other. This facilitates formation of the next peptide bond.
43. A virus takes over the protein-synthesizing machinery of the cell. It uses its own nucleic acids and the cell's ribosomes.
44. The other amino acids found in proteins are created by modifying one of the twenty standard amino acids after the protein is made. Selenocysteine is formed while the amino acid is bound to tRNA. Thus, this amino acid is inserted into the protein during translation just like the standard 20 amino acids are.
45. It is unique chemically because it has a selenium ion in it, which takes the place of a sulfur in cysteine. It is also unique in that appears to be coded in the DNA sequence, even though the codon would normally be a stop codon.

12.5 Eukaryotic Translation

46. Pro-X-Thr is conserved in RF-1 and Ser-Pro-Phe is conserved in RF-2.
47. The sequence Gly-Gly-Gln.
48. Similarities between protein synthesis in bacteria and protein synthesis in eukaryotes: same start and stop codons; same genetic code; same chemical mechanisms of synthesis; interchangeable tRNAs. Major differences: in prokaryotes, the Shine–Dalgarno sequence and no introns; in eukaryotes, the 5′-cap and 3′-tail on mRNA and introns have been spliced out.
49. The original N-terminal methionine can be removed by posttranslational modification.
50. Puromycin would be useful for treatment of a viral infection, but chloramphenicol would not. Viral mRNAs are translated by eukaryotic translation systems, so one must use an antibiotic active on eukaryotic systems.
51. Protein synthesis in prokaryotes takes place as a coupled process with simultaneous transcription of mRNA and translation of the message in protein synthesis. This is possible because of the lack of compartmentalization in prokaryotic cells. In eukaryotes, mRNA is transcribed and processed in the nucleus and only then exported to the cytoplasm to direct protein synthesis.

52. Some mutations can introduce stop codons. It is useful to a cell to have some mechanism to suppress the formation of incomplete proteins.
53. New protein synthesis is involved in long-term memories.
54. A critical role is played by the transcription factor CREB (chapter 11) in turning short-term memories into long-term ones.
55. Animals given drugs that block protein synthesis cannot form new long-term memories, yet their ability to make short-term memories is preserved.
56. The strength, whether due to a single strong impulse or repeated impulses, depolarizes the cell membrane of the nerve. Only ones that are strong enough will be passed along to a receiving neuron. This controls whether a memory can become long-term.

12.6 Posttranslational Modification of Proteins

57. Hydroxyproline is formed from proline, an amino acid for which there are four codons, by posttranslational modification of the collagen precursor.
58. A paper in *Science* showed that in eukaryotic translation, AUG is not always the start codon. In the mammalian immune system, peptides are synthesized for the purpose of presenting them on the surface of the major histocompatibility complexes. The studies found that such peptide synthesis often uses CUG as the start codon instead of AUG. This binds to tRNA-Leu, leaving leucine as the N-terminal amino acid.
59. Peptides of the immune system that are displayed on the surface of the major histsocompatibility complexes
60. Proteins that bind to proteins while they are being synthesized and aid in their correct folding and keep them from associating with other proteins they should not.
61. Protein synthesis would be less efficient and many proteins would be mis-folded and useless.
62. By studying heat shock proteins in bacteria
63. No, it was discovered that the ribosomes themselves aid in protein folding

12.7 Protein Degradation

64. Ubiquitin is a small polypeptide (76 amino acids) that is highly conserved in eukaryotes. When ubiquitin is linked to a protein, it marks that protein for degradation in a proteasome.
65. If proteins to be degraded did not have some signal marking them, the process would take place more randomly and thus be less efficient.
66. If protein degradation took place at any location in a cell, indiscriminate breakdown of functional proteins could take place, so this is an unlikely occurrence. It is much more useful to the cell to have a mechanism for tagging proteins to be degraded and to do so at a specific location in the cell.
67. They are specific sequences that tell the splicing machinery where to splice out introns. A mutation in an ESE could lead to incorrect removal of introns and an entire exon being left out of the final mRNA.
68. A silent mutation is a change in the DNA sequence of a codon that should lead to the same amino acid being inserted. It is a misnomer because we now know that

sometimes differences in codons for the same amino acid do affect the overall protein product.

69. If the silent mutation is in an exonic splicing enhancer, then the splicing out of introns could be incorrect and the correct exon could be skipped.
70. Silent mutations in the mRNA for the enzyme control secondary structures of the mRNA, which controls fast and how often the mRNA is translated, leading to different levels of the enzyme related to pain tolerance.
71. Marfan syndrome, androgen-insensitivity syndrome, cholesteryl ester storage disease, McArdle disease, and phenylketonurea.
72. Hypoxia Inducible Factors (HIF's)
73. When oxygen is low, a prolyl hydroxylase cannot function to hydroxylate a specific proline residue on HIFα. When hydroxylated, the HIFα is targeted for protein degradation. Thus, in low oxygen the HIFα survives long enough to do its job, which is to bind to HIFβ and activate transcription of the genes that produce more red blood cells.